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## EFFECT OF MECHANICAL VIBRATION GENERATED IN OSCILLATING/VIBRATORY PLATFORM ON THE CONCENTRATION OF PLASMA BIOMARKERS AND ON THE WEIGHT IN RATS.

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## Abstract

**Background:** Whole body vibration (WBV) exercise has been used in health sciences. Authors have reported that changes on the concentration of plasma biomarkers could be associated with the WBV effects. The aim of this investigation is to assess the consequences of exposition of 25 Hz mechanical vibration generated in oscillating/vibratory platform (OVP) on the concentration of some plasma biomarkers and on the weight of rats.

**Materials and Methods:** *Wistar* rats were divided into two groups. The animals of the Experimental Group (EG) were submitted to vibration (25 Hz) generated in an OVP with four bouts of 30 seconds with rest time of 60 seconds between the bouts. This procedure was performed daily for 12 days. The animals of the control group (CG) were not exposed to vibration.

**Results:** Our findings show that the WBV exercise at 25 Hz was not capable to alter significantly ( $p < 0.05$ ) the weight of the rats. A significant alteration in the concentrations of amylase was found.

**Conclusion:** Our results indicate a modulation of the WBV exercise with vibration of 25 Hz of frequency (i) in the pathways related to the weight and (ii) in the concentration of some biomarkers, such as amylase.

**Keywords:** plasma biomarkers; mechanical vibration; weight; whole body vibration.

**Abbreviations:** CEUA: Ethics Committee on Animal Experimentation, CG: Control group, CK: creatine kinase, CNPq: Conselho Nacional de Desenvolvimento e Pesquisa, *d*: effect size, DM-2: diabetes mellitus type 2, EG: Experimental group, FAPERJ: Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro, HDL: high density lipoprotein, IBRAG: Instituto de Biologia Roberto Alcântara Gomes, IL: interleukin, LIV: low-intensity vibration, OVP: oscillating/vibratory platform, *p*: level of significance, RT: resistance Training, UERJ: Universidade do Estado do Rio de Janeiro, VLDL: very low density lipoprotein, WBV: whole body vibration,  $\gamma$ GT: gamma glutamyl transferase, <sup>99m</sup>Tc: technetium-99m.

## Introduction

Mechanical vibration is a physical stimulus that can be transmitted to a subject and to generate whole body vibration (WBV) that has been used as a kind of exercise for sports training. Moreover, WBV exercise has been utilized as a modality of non-pharmacological intervention in various clinical interventions (Issurin, 2005; Prisky et al. 2008; Rittweger, 2010; Collado-Mateo et al. 2015), as tool to be used in physiotherapy. WBV exercise is generated due to the exposure of a subject to vibration produced in oscillating/vibratory platform due to the contact of a person with this platform (Rittweger, 2010).

It has been demonstrated in the literature that the WBV exercise improves the walking function (Gómez-Cabello et al., 2013), the muscle strength (Gómez-Cabello et al. 2013; Tankisheva et al. 2014), flexibility (Gómez-Cabello et al. 2013; Sá-Caputo et al. 2014), the bone mineral density (Totossy de Zepetnek et al. 2009; Armbrrecht et al. 2010), the cardiovascular fitness (Bogaerts et al. 2009) and the body balance (Prisby et al. 2008; Torvinen et al. 2002). Furthermore, an increase in the leg muscle force, power, rate of force development and movement velocity has been also reported (Prisby et al. 2008; Russo et al. 2003; Cardinale and Wakeling, 2005; Marín et al. 2009).

Different experimental models have been used to evaluate the effects of the WBV. Rubin et al. (2003) observed a significant increase in femoral trabecular bone mass in adult ewes following one year of vibration as compared to the control group. Christiansen and Silva (2006) have reported that increasing accelerations enhanced trabecular bone volume in a non-dose-dependent fashion as assessed by histo-morphometry in the proximal tibia of adult mice. Pereira et al. (2013) have shown that exposure of animals to WBV can lead to alterations on the uptake of the radiopharmaceutical <sup>99m</sup>Tc-methylenediphosphate in organs, such as the stomach, bowel, kidneys, urinary bladder and prostate. Weinheimer-Haus et al. (2014) investigated a novel therapeutic approach to wound healing-whole body low-intensity vibration (LIV) in diabetic mice. It was concluded that the findings indicate that LIV may exert beneficial effects on wound healing by enhancing angiogenesis and granulation tissue formation, and these changes are associated with increases in pro-angiogenic growth factors.

The mechanisms that are responsible for the effects of the WBV exercise are complex and they are not fully understood. However, Prisby et al. (2008) have suggested direct and indirect effects could be associated with the observed biological consequences of these vibrations. Considering the direct effect, Rittweger (2010) has reported that the body of the subject submitted to WBV has important acceleration and this fact contributes to produce effects. In addition, Prisby et al. (2008) have proposed that the indirect effects of the WBV might be associated with responses of the neuroendocrine system to this mechanical stimulus. Furthermore, some authors have described that repeated muscle contractions might potentially exert endocrine and/or metabolic effects (Marín et al. 2009; Bosco et al. 2000; Di Loreto et al. 2004; Goto and Takamatsu, 2005; Kvorning et al. 2006). In addition, WBV would act through repetitive sensorial-motor stimulation (Ahlborg et al. 2006; Ebersbach et al. 2008).

Studies investigating the effect of WBV on the plasma concentration of biomarkers in human beings have also reported, as the review published by Sá-Caputo et al. (2015). Some hormonal changes, such as an increase (i) in testosterone (Bosco et al. 2000), (ii) in growth hormone (Bosco et al. 2000) and (iii) in epinephrine and norepinephrine have been reported (Di Loreto et al. 2004; Goto and Takamatsu, 2005). Changes in nonhormonal biomarkers have also been reported as the fatty acid concentrations (Goto and Takamatsu, 2005) as well as change in blood glucose levels (Behboudi et al. 2011) have all been demonstrated.

Humphries et al. (2009) divided subjects in WBV, WBV plus resistance training (WBV+RT), or control groups for 16 wk. They found that the subject of the group WBV and WBV plus resistance training experienced a significant ( $P<0.05$ ) increase of 60% and 58% in adiponectin, 48% and 30% in transforming growth factor-beta1, and 17% and 34% in nitric oxide with an accompanying 50% and 36% decrease in osteopontin, 19% and 34% in interleukin (IL)-1beta, and 38% and 39% in tumor necrosis factor-alpha.

Studies with animals have been also carried out by some authors to evaluate the effect of the WBV in the plasma concentration of some biomarkers. Naghii et al. (2011) have found significant differences in plasma levels of creatine kinase (CK), estradiol, and IL-6 between the vibration and control groups of male rats submitted to vibrations with frequencies of 10-50 Hz. The mean vitamin D level was 15% higher and IL-6 level was 32% higher in the group exposed to vibration. Similarly, Naghii and Hedayat (2013) reported a significant increase in plasma levels of xanthine oxidase. Pawlak et al. (2013) have exposed *Wistar* rats to vibration with 50 Hz of frequency for 3 and 6 months respectively. Blood was collected and red and white blood cells, lymphocytes, monocytes, granulocytes, hemoglobin, and hematocrit, as well as IL-1b, IL-10, IL-6, and vascular endothelial growth factor levels were determined. No significant differences between the experimental groups and control group were found for either total blood counts or selected immunological parameters. Monteiro et al. (2015) have reported a significant reduction in gamma glutamyl transferase ( $\gamma$ GT), very low-density lipoprotein (VLDL) and leukocytes after 1-min/day exposure of 20 Hz vibration.

Rubin et al. (2007) have reported that 15 weeks of brief, daily exposure to high-frequency mechanical signals, induced at a magnitude well below that which would arise during walking, inhibited adipogenesis by 27% in C57BL/6J mice. The mechanical signal also reduced key risk factors in the onset of type II diabetes, nonesterified free fatty acid and triglyceride content in the liver, by 43% and 39%, respectively. They pointed out that these findings translated to humans may represent the basis for the nonpharmacologic prevention of obesity and its sequelae.

In addition, Rittweger (2010) has pointed out that there is evidence of the involvement of muscular contractions during WBV. Rittweger et al. (2002) have demonstrated that the metabolic power in whole-body can be controlled by biomechanical parameters as frequency and peak-to-peak displacement, and by application of additional loads. Rauch et al. (2010) have published guidelines with several biophysical considerations about the use of the mechanical vibrations generated in oscillating/vibratory platform, as the frequency and the peak to peak displacement of the vibration.

Putting together the findings described, it is possible to verify that more research is necessary to try to better understand the effect of the vibrations generated in the oscillating/vibratory platform in the biological system. In addition, as these effects seem to be associated with changes in the concentration of some biomarkers, the purpose of this study is to determine the effect of 25 Hz vibration generated by an oscillating/vibratory platform on some biochemical blood markers and on the weight of rats. Moreover, the findings obtained in this work will assist further research on optimal parameters for the WBV exercise.

## Material and Methods

### Animals

Adult male Wistar rats (n=12, 2 months old) were maintained in an ambient with controlled light (12:12 h light/dark) and temperature ( $25 \pm 2$  °C) in the *Biotério Central* of the *Instituto de Biologia Roberto Alcântara Gomes (IBRAG)*, *Universidade do Estado do Rio de Janeiro (UERJ)*, Brazil. Food (Nuvital, Colombo, PR, Brazil) and water were provided *ad libitum*. The experimental model was approved by the Ethics Committee on Animal Experimentation of the IBRAG, UERJ under the reference CEUA/IBRAG/UERJ number 041/2013).

The rats were divided in control (CG) (n=6) and experimental group (EG) (n=6) and the weight of the animals was determined before the first and after the last session of a protocol in the oscillating/vibratory platform.

### Characteristics of the oscillating/vibratory platform

The platform used in the experiment was an oscillating system (Novaplate fitness evolution, DAF, *Produtos Hospitalares Ltd*, São Paulo, Brazil). In this device, there is a reciprocating vertical displacement on the left and right side of a fulcrum. When the right side of the base of the platform is going down, the left side is going up, and vice-versa (Rittweger, 2010). It is a side-alternating vibration system working as a teeterboard (28 width and 58 cm length) with amplitude of 0 (zero) mm in the center of the platform up to the maximum in the edge that was 7.5 mm. The animals of the EG were put in a man-made acrylic base fixed in the teeterboard of the platform with a tape, as it is shown in the Figure 1. The frequency of the vibrations used was 25 Hz and the media displacement peak to peak was 4 mm.

### Experimental procedure

The animals of the experimental group were in an acrylic base that was fixed with a tape (Figure 1) on the base of the platform and were submitted to sessions with four bouts of 25 Hz mechanical vibration for 30 seconds with a rest of 60 seconds between the bouts. In this investigation, a total time per day similar as reported by Pawlak et al. (2013) was followed. This procedure was performed daily for 12 consecutive days. The animals of the control group (CG) were not exposed to the mechanical vibration. The animals of this group were in a cage that was close to the platform, however there was not a contact with it.

After the 12<sup>th</sup> day, the rats of the CG and EG were anesthetized with sodium thiopental and aliquots of blood of all rats were taken by cardiac puncture and stored in tubes with EDTA anticoagulant. The biochemical analysis was performed with specific kits. The concentration of selected biomarkers was measured in a clinical laboratory of the *Universidade do Estado do Rio de Janeiro*. The biomarkers evaluated in the control group (CG) and in the experimental group (EG) were glucose, creatinine, total cholesterol, triglycerides, HDL (high density lipoprotein), bilirubin, CK, total protein, albumin, alkaline phosphatase, lipase, amylase, calcium and magnesium. The determinations were performed in automated equipment (COBAS INTEGRA 400 plus, Roche, Basel, Switzerland).

The weight of the animals of the CG and EG was determined just before the first session and after the last session in a commercial balance.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation. The unpaired Mann-Whitney test was performed to results concerning to the concentration of the biomarkers. A *t*-test was used to compare the weight of the animals before and after the treatment. A *p*-value of less than 0.05 was considered statistical significance. The Cohen's (*d*) effect sizes were used to determine the magnitude of the effect independent on the sample size. Cohen's test (*d*) were calculated using the formula (Cohen, 1988):

$$d = \frac{M1 - M2}{\sqrt{\frac{SD1^2 + SD2^2}{2}}}$$

A  $d$ -value less than  $\leq 0.2$  was considered a small effect sizes, between  $0.2 < d < 0.8$  a moderate effect sizes and  $d \geq 0.8$  large effects sizes.

## Results

In Table 1 the weight of the animals of the CG and EG before and after the procedure involving WBV exercise with vibration of 25 Hz is shown. It is possible to verify that the weight of the animals of the CG has significantly ( $p < 0.05$ ) increased. However, the weight of the rats of the group that has been submitted to the WBV exercise with frequency of 25 Hz has not increased significantly ( $p > 0.05$ ). Considering the Cohen's effect sizes, the finding of the control group is large, while of the experimental group is moderate.

**Table 1:** The weight of the animals of the CG and EG before and after the procedure involving WBV exercise with vibration of 25 Hz

Groups	Weight before (g)	Weight after (g)	$p$ -value	$d$ -value
CG	230.0 $\pm$ 19.4	251.6 $\pm$ 19.3*	0.0409	1.11
EG	223.3 $\pm$ 22.8	240.7 $\pm$ 23.1	0.1103	0.75

CG – Control group, EG – Experimental group,  $p$  – level of significance, \*statistical difference ( $p < 0.05$ ),  $d$ -value - Cohen's effect sizes,  $d \leq 0.2$  was considered a small effect sizes,  $0.2 < d < 0.8$  a moderate effect sizes and  $d \geq 0.8$  large effects sizes. The weight of the animals was determined before and after the WBV exercises.

In Table 2 is shown the plasma concentration of some biomarkers of the animals of the CG and EG before and after the procedure involving WBV exercise. It is demonstrated that the concentration of the almost all the evaluated biomarkers were not significantly altered due to the WBV exercise with vibration of 25 Hz. A significant ( $p \leq 0.05$ ) decrease was found only on the concentrations of amylase. In addition, the Cohen's effect sizes related to this biomarker was higher than 0.8, which is a large effect size.

**Table 2:** Concentration of some biomarkers determined in animals submitted to the 25 Hz vibration generated in oscillating platform.

Biomarkers	CG	EG	$p$ -value	$d$ -value
	Mean $\pm$ SD	Mean $\pm$ SD		
Glucose (mg/dL)	149.3 $\pm$ 50.4	125.6 $\pm$ 20.5	0.42	0.61
Creatinine(mg/dL)	0.66 $\pm$ 1.04	0.24 $\pm$ 0.05	0.32	0.57
Cholesterol (mg/dL)	54.16 $\pm$ 9.45	55.6 $\pm$ 4.39	0.5	0.19
Triglyceride (mg/dL)	48.16 $\pm$ 18.61	34.6 $\pm$ 13.83	0.13	0.82
HDL (mg/dL)	43.66 $\pm$ 7.14	45.14 $\pm$ 3.84	0.29	0.25
Alkaline Phosphatase (U/L)	183 $\pm$ 16.80	165 $\pm$ 24.89	0.13	0.84
Bilirubin (mg/dL)	0.05 $\pm$ 0.02	0.05 $\pm$ 0.01	0.35	0.00
Amylase (U/L)	2979 $\pm$ 594.68	2360.8 $\pm$ 234.83	0.03*	1.36
Lipase (U/L)	7.13 $\pm$ 0.57	7.18 $\pm$ 0.54	0.29	0.09
CK (U/L)	3456.1 $\pm$ 4436.7	3710.4 $\pm$ 5448.4	0.42	0.08
Calcium (mg/dL)	10.95 $\pm$ 0.88	10.02 $\pm$ 0.68	0.051	1.18
Magnesium (mg/dL)	3.08 $\pm$ 0.76	2.78 $\pm$ 0.30	0.29	0.51
Total Protein(mg/dL)	5.58 $\pm$ 0.52	5.4 $\pm$ 0.25	0.23	0.44
Albumin (mg/dL)	3.65 $\pm$ 0.27	3.56 $\pm$ 0.05	0.13	0.46

CG – Control group, EG – Experimental group,  $p$  – level of significance, \*statistical difference ( $p < 0.05$ ),  $d$ -value - Cohen's effect sizes,  $d \leq 0.2$  was considered a small effect sizes,  $0.2 < d < 0.8$  a moderate effect sizes and  $d \geq 0.8$  large effects sizes. The weight of the animals was determined before and after the WBV exercises.

## Discussion

The use of physical stimulus is common in health sciences; and WBV exercises generated by the mechanical vibrations produced in oscillating/vibratory platform been used in several approaches (Rønnestad, 2009). The results of this study show that exposition during 12 days to mechanical vibration at 25Hz prevents an increase of the weight in the animals. Otherwise, the weight of the animals of the control group has significantly increased. Moreover, 25 Hz vibration was not capable in affecting the concentration of some biomarkers in the blood of rats, with exception to the amylase.



There is evidence of the muscular contractions during WBV exercise could be explained by a direct effect of the vibrations (Zaidell et al. 2013). In consequence, this activity of the muscle would lead to the consumption of energy (Serravite et al. 2013). Wang and Kerrick (2002) have verified that the application of vibration to intact or skinned single fiber preparations determine increases in ATP turnover. In addition, authors (Rittweger, 2010; Rittweger et al. 2001) have reported that in vibrations with frequency of 26 Hz, with and without an additional load there is an energy turnover that amounts to 4.5 ml. min<sup>-1</sup>.kg<sup>-1</sup>. The findings shown in Table 1 are in agreement with these considerations due to the weight of the animals of the control group (not submitted to vibration) has significantly ( $p<0.05$ ) increased, while the weight of the rats of the group that has been submitted to the WBV exercise was not increased. Hazell and Lemon (2012) have observed that a day with a 30-min multiple exercise (five, 30 s, 15 repetition sets of six exercises; 1:1 exercise: recovery ratio), WBV session increased 24 h VO<sub>2</sub> versus a day that included the same exercise session without vibration, and versus a non-exercise day by 10 and 25%, respectively. In addition, Rubin et al. (2007) have reported that exposure to high-frequency mechanical signals inhibited adipogenesis by 27% in C57BL/6J mice. It is interesting to suggest that the WBV exercise with the frequency of 25 Hz would be capable of modulate the pathways of the metabolism that are related with the increase of the weight, as controlling the ATP turnover. Erceg et al. (2015) have reported that although bone metabolism was altered by WBV training, no associations were apparent between osteocalcin and insulin resistance. These findings suggest WBV exercise may positively increase body mass index and bone mineral density by decreasing bone resorption in overweight boys. Interestingly, Rittweger et al. (2010) has asked a question in a very interesting and suitable revision about the effect of the WBV exercises: "Can this kind of energy turnover help to reduce body weight?"

In Table 2 is shown the plasma concentration of some biomarkers of the animals of the CG and EG before and after the procedure involving WBV exercise. It is demonstrated that the concentration of the almost all the evaluated biomarkers were not significantly altered due to the WBV exercise with vibration of 25 Hz. It was found only significant ( $p\leq 0.05$ ) decrease in the plasma concentrations of the amylase. In addition, the Cohen's effect sizes related to these biomarkers was higher than 0.8, which is a large effect size. Goto and Takamatsu (2005) have reported that WBV exercises increase the concentration of the free fatty acids in the plasma indicating that the vibration is capable of interfering with lipolysis. In disagreement, Naghii and Hedayati (2013) using a protocol with vibration with a frequency of 10–50 Hz have observed that the weight of animals submitted to the vibration increased. The consideration of this finding might be important for further research in the obese population.

Although some authors have reported that the WBV exercises could reduce glucose levels in patients with diabetes mellitus type 2 (Behboudi et al. 2011), our findings revealed no change in the concentration of this biomarker in the blood of rats. This may however be because this study was conducted in healthy animals. Monteiro et al. (2015) have reported a no significant alteration in the glucose after 1-min/day exposure of 20 Hz vibration. Di Loreto et al. (2004) reported in their study that vibration at 30 Hz of frequency resulted in a slight reduction in blood glucose levels in patients with DM-2. These findings suggest that for this population (patients with DM-2) exposure to vibration a higher frequency may be beneficial. However, whether this effect can be attributed to vibration or exercise, still needs to be tested.

The effects of 25 Hz vibration on the levels of some biomarkers of lipid metabolism in the blood also varied. Although Goto and Takamatsu (2005) found an increase in serum fatty acids in healthy individuals subjected to vibration by exercising on a vibrating platform, the results presented in Table 2 show no alteration in the concentration of the lipids that were evaluated. Naghii et al. (2011) have not found alterations on the concentration of these biomarkers. Monteiro et al. (2015) have reported no significant alteration in the concentration of total cholesterol, triglycerides, HDL, LDL and VLDL, although a significant reduction in VLDL after 1-min/day exposure of 20 Hz vibration during seven days was observed.

No significant differences were found regarding the urea, creatinine and lipase concentrations following exposure to 25 Hz vibration (Table 2) and these findings are in agreement with Naghii et al. (2011).

A decrease in the concentration of the amylase was found. The interpretation of this result is not simple, but as the serum amylase and lipase are common tests obtained as biochemical markers for acute pancreatitis (Chase et al. 1996), it is possible to suggest a possible interference of the 25 Hz vibration in the pancreas. Moreover, as amylase is an enzyme which helps in the digestion of glycogen and starch (PubMed Health, 2015), the decrease of its concentration due to the vibration would aid in the maintenance of the weight (Table 1) of the rats.

Among some limitations of this current investigation, it is important to point out that the rats of the experimental group were in an acrylic base that was fixed with a tape at the base of the platform, whereas the rats of the control group were not fixed (they were free in a cage). The number of animals in each group was other limitation. Despite this experimental condition, important findings were obtained.

In conclusion, analysis of our results indicates a modulation of the WBV exercise with vibration of 25 Hz of frequency (i) in the pathways related to the weight and (ii) in the concentration of some biomarkers, such as amylase.

Competing interests

The authors declare that in this study there is no any competing interests.

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